Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

Listing of Claims

1. (currently amended) A yeast which ferments xylose to ethanol, comprising:

genes integrated at ~~each of~~ multiple reiterated ribosomal DNA sites of the yeast, said genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase , wherein fermentation activity is not decreased after culture in non-selective medium for greater than 40 generations.

2. (original) The yeast of claim 1 which also ferments glucose to ethanol.

3. (original) The yeast of claim 2 which is Saccharomyces.

4. (original) The yeast of claim 3 wherein said sites are non-transcribed DNA sites.

5. (original) The yeast of claim 1 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

6. (original) The yeast of claim 5 wherein the promoters do not require xylose for induction.

7. (original) The yeast of claim 3 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol.

8. (original) The yeast of claim 4 wherein the genes are fused to non-glucose-inhibited promoters and the yeast simultaneously ferments glucose and xylose to ethanol, the promoters also not requiring xylose for induction.

9. (original) The yeast of claim 6 wherein the xylose reductase and xylitol dehydrogenase genes are from natural yeast which ferment xylose to ethanol.

10. (currently amended) The yeast of claim 9 wherein the natural yeast are Candida ~~Shehatae~~ shehatae, Pichia stipitis or Pachysolen tannophilus.

11. (original) The yeast of claim 9 wherein the xyulokinase gene is from a yeast or bacteria.

12. (currently amended) The yeast of claim 11 wherein the xyulokinase gene is from Candida ~~Shehatae~~ shehatae, Pichia stipitis, Pachysolen tannophilus, Saccharomyces cerevisiae, Schizosaccharomyces pombe, or Escherichia coli.

13. (previously presented) The yeast of claim 1 having said genes integrated at least about 10 ribosomal DNA sites of the yeast.

14. (currently amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(a) transforming the cells with a replicative and integrative plasmid comprising [[an]] a yeast autonomous replicating sequence, exogenous DNA, and a first selection marker; and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of

the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

15. (original) The method of claim 14, wherein the plasmid DNA also includes a second selection marker for selecting cells which include the plasmid.

16. (currently amended) The method of claim 14 wherein the cells are yeast or eukaryotic cells, and wherein the method further includes the step of repeatedly replicating the progeny cells from step (b) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover ~~yeast~~ cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

17. (original) The method of claim 16 wherein the cells are yeast cells and the exogenous DNA includes genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, which also serve as the first selection marker.

18. (currently amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(i) transforming yeast cells with a replicative and integrative plasmid comprising [[an]] a yeast autonomous replicating sequence, exogenous DNA comprising genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, wherein the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, and a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly replicating the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the

progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA , wherein the progeny cells ferment xylose to ethanol; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

19. (currently amended) Yeast cells produced by the method of claim 18 , wherein fermentation activity of the yeast cells of step (iii) is not decreased after culture in non-selective medium for greater than 40 generations.

20. (canceled)

21. (currently amended) The yeast cells of claim ~~20~~ 19, wherein said genes are fused to non-glucose-inhibited promoters which do not require xylose for induction, and wherein the yeast cells ferment glucose and xylose simultaneously to ethanol.

22. (canceled)

23. (currently amended) A yeast which ferments xylose to ethanol, comprising:

multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase fused to non-glucose inhibited promoters, the yeast fermenting glucose and xylose simultaneously to ethanol ~~and substantially retaining its capacity for fermenting xylose to ethanol for at least 20 generations when cultured under non-selective conditions~~ , wherein fermentation activity is not decreased after culture in non-selective medium for greater than 40 generations.

24. (original) The yeast of claim 23, wherein said promoters do not require xylose for induction

25. (currently amended) A yeast which ferments xylose to ethanol, comprising:

multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, the yeast fermenting xylose to ethanol ~~and substantially retaining its capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least 20 generations~~ , wherein fermentation activity is not decreased after culture in non-selective medium for greater than 40 generations.

26. (currently amended) The yeast of claim 25, wherein the promoters do not require xylose for induction .

27. (previously presented) A method for fermenting xylose to ethanol, comprising fermenting a xylose-containing medium with a yeast of claim 1, 22, 23, 24, 25, or 26, to produce ethanol.

28. (currently amended) A plasmid vector comprising a functional yeast autonomous replicating sequence and an exogenous DNA comprising a first selection marker, the exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further including a second ~~section~~ selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell.

29. (previously presented) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose

reductase, xylitol dehydrogenase, and xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of the target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol.

30. (currently amended) A method for producing cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising [[an]] a yeast autonomous replicating sequence and containing the exogenous DNA to produce multiple generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

31. (canceled)

32. (previously presented) The method of claim 14 wherein the cells are yeast.

33. (previously presented) The method of claim 30 wherein the cells are yeast.

34. (previously presented) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell.